Heritabilities of Ocular Biometrical Traits in Two Croatian Isolates with Extended Pedigrees

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Purpose. To assess the effects of body stature and years of education, in addition to age and sex, on six oculometric traits and to estimate the heritabilities of these quantitative traits in two Croatian cross-population studies.

Methods. Adult subjects living on the two Croatian islands of Vis and Korčula were recruited for a large epidemiologic and genetic study that included eye biometry, keratometry, and autorefraction. Effects and heritabilities were estimated by using general linear mixed models for axial length (AL), anterior chamber depth (ACD), corneal curvature (CC), corneal thickness (CT), lens thickness (LT), and spherical equivalent refraction (SER). Both cohorts were genotyped with dense SNP arrays, allowing the use of kinship coefficients derived from genotypic data (realized kinship) rather than from pedigree information (expected kinship).

Results. Across cohorts, body mass index (BMI) did not consistently influence any of the ocular traits adjusted for age and/or sex, whereas height and years in education (YrEd) did, explaining up to an additional 5% of the variance (in CC). CT was the trait least influenced by covariates. Estimated heritabilities in Vis and Korčula, respectively, were 84% and 52% for CC, 75% and 71% for CT, 37% and 32% for LT, 59% and 45% for ACD, 37% and 74% for AL, and 0% and 17% for SER.

Conclusions. While heritabilities of CT and CC seemed uniformly high across studies of Caucasian datasets, estimates for SER varied widely and were at the lower end of the spectrum of published observations in our study. (*Invest Ophthalmol Vis Sci.* 2010;51:737–743) DOI:10.1167/iovs.09-3720

Studying quantitative endophenotypes was advocated to help unravel the genetic architecture of common diseases.[1,2] Successes met by this approach include mapping of genes modulating QT elongation measured by ECG and cardiac arrhythmia risk,[3] IgE levels and asthma risk,[4] serum uric acid level and gout risk,[5] and lipid levels and coronary heart disease risk.[6] Ocular conditions, in particular the most common one, refractive error, lend themselves very well to this approach. Myopia and hypermetropia can be viewed largely as defects in the eye growth processes that normally adjust AL of the eye to the optical power of the cornea and lens. The values of the separate refractive components (axial length [AL], power of the cornea, and power of the lens), which if uncoordinated lead to refractive errors, have long been recognized as being normally distributed in general population surveys, whereas the distribution of refraction itself has a greater density around emmetropic values.[7] Researchers in several large studies of unselected individuals, predominantly twins, have investigated to what extent genetic variation contributes to ocular quantitative components, and results have generally supported a substantial polygenic contribution. These include reports on AL, anterior chamber depth (ACD), corneal curvature (CC), and spherical equivalent refraction (SER) in a Sardinian isolate (n = 741; mean age, 41 years)[8]; in the Australian GEM twin study (n = 1224; mean age, 52 years)[9]; and in a Danish twin cohort (n = 114; age range, 20–45 years)[10] together with lens thickness (LT), and analysis of refraction alone in a UK female twin cohort (n = 506; mean age, 62.4 years)[11] and in the Beaver Dam population study (n = 2138; age range, 43–84 years).[12] For corneal thickness (CT) there is, to our knowledge, only one previous report of heritability, 95% in a European sample of UK and Australian twins (n = 256; mean age, 38 years).[13] This trait is now a recognized risk factor for progression from ocular hypertension to primary open-angle glaucoma,[14] as well as a determinant of corneal refractive power.

There has been a call for caution regarding the high heritabilities reported for refraction and AL from twin studies, ranging from 75% to 94%,[9,11] in view of the much lower heritabilities, 18%[8] to 34%,[12] obtained from parent–offspring correlations.[15] Heritability estimates, in both twin and family studies depend on different assumptions and are likely to be divergent for traits strongly influenced by environmental cues, such as myopia.[15,16] Cross-population studies in isolated populations offer the advantage of accessing large complex pedigrees where heritabilities can be drawn simultaneously from the comparison of multiple pairs of relatives. They also benefit

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from a more stable and uniform diet, climate, and living conditions. However, oculometric traits were analyzed in only a few of those studies.[8] The resemblance between distant relatives is less likely to be biased by nongenetic factors, but their genetic covariance is typically small and not well estimated if based on pedigree knowledge only (due to the stochasticity of segregation and recombination). In the present study, we measured the heritability of six ocular biometric traits in two isolated Croatian populations based on realized co-ancestry coefficients drawn from molecular marker information, allowing better estimates of true sharing and thus of heritability. Because of the ethnic variations in ocular morphology, we compared our results only with data derived from populations of European descent.

Although numerous studies have shown that ocular biometry can be affected by the amount of near work,[17,18] it has also been hypothesized that a diet rich in processed foods plays a role in the increase in juvenile-onset myopia.[19] The extent to which body stature and level of education contribute to the values of the traits analyzed was therefore examined. To a large extent, these covariates, including educational achievement,[20] are themselves known to have a strong genetic component.

Although heritabilities are population specific in principle, in practice they are very similar across populations for morphometric traits and are usually high.[21] Estimates of trait heritability in our study should thus inform on the contribution of genetic variants underlying these traits in the studied populations as well as others and guide the choice of covariates to take into account for follow-up gene-mapping studies.

METHODS

Subjects

Adult subjects living on the two Croatian islands of Vis and of Korčula were recruited for large, population-based, genetic studies, in Spring 2003 and Spring 2004 on Vis and in Spring and Autumn 2007 on Korcula. The studies received approval from the relevant ethics committees in Scotland and Croatia and complied with the tenets of the Declaration of Helsinki. All participants were volunteers and gave informed consent. They underwent a medical examination and interview, led by research teams from the Institute for Anthropological Research and the Andrija Stampar School of Public Health, (Zagreb, Croatia). All subjects visited the clinical research center in the region, where they were examined in person and where fasting blood was drawn and stored for further analyses. Biochemical and physiological measurements were performed, and questionnaires of medical history as well as lifestyle and environmental exposures were collected.

Island of Vis

The Vis study included 1030 unselected adult participants, aged 18–93 years (mean, 56), a subset of which ($n = 640$) underwent a complete eye examination in summer 2007 and provided an ophthalmic history. Examinees were recruited on the basis of the electoral register, which lists the persons who are permanently living on the island, as opposed to the official census which tends to overestimate the island's true population. A postal invitation was sent to all registered individuals. The response rate (70%) was high. Genealogical records were reconstructed based on church parish records, as well as information provided by participants, and 588 participants could be placed in 125 pedigrees. (The median number of participants linked per pedigree was 2, but 10 pedigrees linked more than 10 individuals, and the largest linked 134 individuals and had a depth of six generations.) This pedigree information, going back to the 1830s, did not reveal any inbreeding loop.

Island of Korčula

The field work was performed in the eastern part of the island, targeting healthy volunteers from the town of Korčula and the villages of Lumbarda, Žrnovo, and Račišće. Recruitment was secured through invitations by mail, posters, radio, and personal contacts. A total of 969 examinees, aged 18 to 98 (mean, 56.3) years, were included in the study, and most ($n = 930$) underwent a complete eye examination. In-depth genealogical research was not performed in this population.

Eye Examination and Measurements

Keratometry (CC) and noncycloplegic autorefraction were measured on each eye with a hand-held autorefractometer/keratometer (Ark30; Nidek, Gamagori, Japan). Refraction was analyzed as the SER (sphere+half the cylinder). CC was the average of the values of corneal radii of curvature from the two principal meridians.

Biometry measurements, AL, ACD, CT, and LT, were performed with an A-scan device (Echoscan US-1800; Nidek). For the A-scan, which required contact with the cornea, oxybuprocaine anesthetic sterile eye drops (Minims; Chauvin Pharmaceuticals, Ltd., Romford, UK) were used.

Measures of eyes with a history of trauma or LASIK or that were aphakic were removed. Right eye values were plotted against the left eye values, and discordant individuals were checked. In most cases, one of the eyes measured was an extreme outlier (more than three times the interquartile range away from the lower or upper quantile) and the data were excluded. Pearson correlations for right and left eyes were all statistically significant (two-tailed significance level of 0.01) for SER (0.8, Korčula and Vis), AL (0.8, Korčula; 0.9, Vis), CC (0.8, Korčula; −0.9, Vis), CT (0.9, Korčula and Vis), LT (0.5, Korčula; 0.6, Vis), and ACD (0.6, Korčula; 0.7, Vis).

Given the high correlations between right and left eye measures, the analysis was performed on the right eye measures, unless the left eye had more complete measurements (e.g., due to trauma or cataract surgery on the right eye).

Genotyping and Quality Control

A large subset of participants were genotyped with a dense SNP array according to the manufacturer's standard recommendations (Beadchip; Illumina Corp., Austin, TX; HumanHap 300 v1 for Vis, HumanCNV370-Duo for Korčula; genotypes were determined with Illumina BeadStudio software). Samples with a call rate below 97% (for SNP of call rate above 98%, a minor allele frequency above 2%, and probability for exact test of Hardy-Weinberg equilibrium above 10^{-10}), and ethnic outliers based on principal components analysis of genotypic data were excluded from the analysis by using the quality control algorithm implemented in a genome-wide SNP analysis program, GenABEL.[1]

After this quality-control step, the number of individuals available with ocular measures and genotypes was 601 in Vis and 859 in Korčula.

Relatedness between participants was estimated from whole-genome data, by using the sharing of genome identical by descent (IBD) estimation function implemented in PLINK, a toolset for whole genome analysis.[22] This method is robust to pedigree information errors, undeclared relationships, and samples swaps and gives realized sharing rather than an expectation based on pedigree information (for the same pedigree-based relationship, realized genome-sharing from a common ancestor varies due to segregation and recombination stochasticity). Using this function, the 859 Korčula samples (601 Vis samples) analyzed consisted of 136 (90) parent-child pairs, 93 (61) sib pairs, 118 (78) avuncular or half-sib pairs, 330 (235) pairs with IBD sharing consistent with first-cousin relationship, 1657 (1290) pairs with first-cousin once-removed levels, and 8150 (5259) pairs with second-cousin levels. The mean IBD sharing between all possible pairs of individuals was 0.003 (min, 0; max, 0.61) in Korčula and 0.004 in Vis (min, 0; max, 0.594). In Vis, close relationships were in complete agreement with the researched pedigree information: the 89 known parent-child pairs for which the expected IBD sharing is 0.5 exactly, had mean calculated IBD sharing of 0.5 (min, 0.5; max, 0.52); the 61

TABLE 1. Descriptive Statistics of Oculometric Traits, Age, and Stature in the Two Croatian Cohorts

	All			Women			Men		
	n	Range (Min–Max)	Mean (SD)	*n*	Range (Min–Max)	Mean (SD)	*n*	Range (Min–Max)	Mean (SD)
Vis Island									
Age, y	601	18–86	56.32 (14.2)	360	18–86	56.2 (14.89)	241	18–80	56.5 (13.3)
Height, cm	600	143–203	167.8 (9.6)	361	143–181	161.9 (6.6)	239	158.2–203.5	176 (7)
BMI, kg/m^2	599	17.01–43.6	27.3 (4.1)	360	17.01–43.6	27.28 (4.39)	239	18.36–40.69	27.34 (3.7)
YrEd, y	598	2–20	10 (3.4)	358	2–16	9.3 (3.3)	240	4–20	11.05 (3.2)
AC, mm	591	2.12–4.37	2.997 (0.4)	355	2.12–4.37	2.96 (0.36)	236	2.19–4.33	3.05 (0.4)
AL, mm	588	19.11–27.89	23.13 (1.02)	352	19.11–27.82	22.92 (0.99)	236	20.12–27.89	23.45 (0.97)
CC, mm	597	6.97–9.19	7.74 (0.3)	358	6.97–8.71	7.69 (0.24)	239	7.08–9.19	7.8 (0.3)
CT, μm	596	445–670	561.2 (34.6)	356	445–658	562.6 (32.64)	240	458–670	559.2 (37.3)
LT, mm	588	3.31–6.01	4.35 (0.4)	352	3.37–5.98	4.33 (0.42)	236	3.31–6.01	4.38 (0.5)
SER, D	571	−14.68–+9.68	−0.21 (2.1)	343	−10.55–+9.68	−0.12 (2.08)	228	−14.69–+6.43	−0.34 (2.02)
Korčula Island									
Age, y	859	18–98	56.2 (13.7)	556	18–98	55.45 (13.4)	303	20–90	57.5 (14.35)
Height, cm	846	140.5–197	167.9 (9.2)	550	140.5–186	163.3 (6.6)	296	158.3–197	176.6 (6.6)
BMI, kg/m^2	846	16.6–53.84	27.96 (4.14)	550	16.59–53.84	27.56 (4.4)	296	19.25–40.67	28.7 (3.6)
YrEd, y	842	1–22	10.8 (3.3)	546	1–22	10.5 (3.4)	296	1–18	11.3 (3.1)
AC, mm	849	2–5.83	2.88 (0.44)	551	2–5.83	2.85 (0.42)	298	2–5.34	2.94 (0.46)
AL, mm	848	17.05–30.68	23.21 (1.12)	551	20.16–30.68	23.03 (1.1)	297	17.05–28.88	23.55 (1.1)
CC, mm	839	7–9.31	7.77 (0.27)	544	7–8.57	7.73 (0.26)	295	7–9.31	7.85 (0.28)
CT, μm	849	457–700	555.6 (35.98)	551	457–700	554.6 (36.5)	298	467–671	557.4 (34.9)
LT, mm	849	3–6.54	4.31 (0.47)	551	3–5.66	4.3 (0.47)	298	3–6.54	4.34 (0.48)
SER, D	836	−15.84–+8.62	−0.25 (1.9)	546	−15.84–+8.62	−0.18 (2)	290	−8.43–+5.5	−0.38 (1.75)

known full sib pairs with expected mean IBD sharing of 0.50 (min, 0.36; max, 0.70) had a calculated mean of 0.50 (min, 0.42; max, 0.59); and the 55 declared avuncular/grandparent-child/half-sib relationships with expected mean 0.25 (0.18–0.35) had a calculated mean IBD of 0.25 (min, 0.17; max, 0.34).

Statistical Analysis

Descriptive statistics and tests were performed with one of two programs (R (http://www.r-project.org; or SPSS, ver. 13; SPSS, Chicago IL). Inverse normal transformation was used to convert SER, ACD, and AL to normal distributions by using the rank transformation function of GenABEL.[23]

Effects of cofactors/covariates and variance components were estimated by maximum likelihood in the classic animal model,[24] a general linear mixed model. Sex, age, height, BMI, and YrEd were tested as fixed effects, with an additive polygenic genetic effect and a residual effect fitted as random effects. This model is the base model of choice for quantitative trait heritability estimation when phenotypic values can be correlated in pairs of individuals of multiple relationships.[25] Models were implemented by using the polygenic function of GenABEL.[26] The pair-wise kinship coefficients, elements of the kinship matrix fitted to account for all relatedness within the sample, were estimated from the genomic data using the gkin function of the statistical package GenABEL.[23]

The statistical significance of a fixed effect or an estimated variance component was determined by a likelihood ratio test (LRT), in which the likelihood for the full model was compared with the likelihood of the nested model, in which the component tested was constrained to be 0.[25] For fixed-effects selection, the best model was chosen based on the most parsimonious model, using the Aikeke information criteria (AIC), $2k - 2\ln(L)$ where k is the number of parameters in the model and L the maximum likelihood of the model.[27] Z-scores[28] were used to test for significant differences between male and female estimates or between Vis and Korčula estimates:

$$Z = (x_i - x_j)/(\sigma_i^2 + \sigma_j^2)^{0.5}$$

where x_i is one estimate of heritability, x_j is the other, and σ_i^2 and σ_j^2 are their respective standard errors. The z-scores were then tested against a large sample standard normal distribution.

RESULTS

Descriptive Statistics

The descriptive statistics of the six oculometric traits measured in the Croatian participants (for which both quality-controlled phenotypic and genotypic data were available) are displayed in Table 1. There were no statistically significant differences in trait mean values between the two isolated populations sampled. The range and mean values were, by and large, similar to those reported in unselected adult populations of European descent in the United Kingdom,[7,11,13] Sardinia,[8] Denmark,[10] Australia,[9] and the United States.[29] The Croatian isolates displayed, on average, a slightly shorter eye and ACD (AL mean of 23.1–23.2 mm rather the mean of 23.4–23.5 mm in these published European datasets) and thicker lenses (4.3 mm compared with the only available data, ~3.9 mm, in a Danish cohort). The distributions of the ocular traits were also very similar to those in the published data from various populations: clearly or nearly Gaussian for CT, CC, ACD, and LT (following the Anderson-Darling normality test implemented in R), with a non-Gaussian excess crowding around the mean for refraction, and to a lesser extent for AL. In both the Croatian islands, there were statistically significant sex differences in mean trait values for CC ($P < 10^{-3}$), AL ($P < 10^{-3}$), and ACD ($P = 0.002$, Vis; $P = 0.004$, Korčula) but not for the other ocular traits.

Phenotypic correlations between ocular traits, adjusted for age and sex and accounting for participants' relatedness, are displayed in Table 2. The strongest correlations were highly significant ($P < 10^{-2}$) in both populations: negative between refraction and AL (−0.483, Vis; −0.594, Korčula) and between refraction and ACD (−0.156, Vis; −0.154, Korčula) and positive between AL and CC (0.455, Vis; 0.435, Korčula) and between AL and ACD (0.331, Vis; 0.367, Korčula). Most remaining trait pairs displayed weaker phenotypic correlations, which were generally significant only in Korčula, where the power of detection was stronger because of the larger sample size. Three trait pairs did not correlate significantly in either population, all including CC with either refraction, ACD or LT.

TABLE 2. Phenotypic Correlations between Oculometric Traits Adjusted for Age, Sex, and Relatedness

Trait	AC Depth	AL	CC	CT	LT	SE Refraction
ACD		**0.331** ($P = 1.3\ 10^{-15}$)	−0.023 ($P = 0.57$)	0.0496 ($P = 0.26$)	**−0.115** ($P = 5.3\ 10^{-3}$)	**−0.156** ($P = 1.8\ 10^{-4}$)
AL	**0.367** ($P = 6.6\ 10^{-16}$)		**0.455** ($P < 2.2\ 10^{-16}$)	0.048 ($P = 0.24$)	−0.035 ($P = 0.4$)	**−0.483** ($P < 2.2\ 10^{-16}$)
CC	0.059 ($P = 8.7\ 10^{-2}$)	**0.435** ($P = 4.4\ 10^{-16}$)		**0.147** ($P = 3.2\ 10^{-4}$)	0.016 ($P = 0.69$)	0.031 ($P = 0.46$)
CT	**−0.077** ($P = 2.\ 10^{-2}$)	**−0.087** ($P = 1.1\ 10^{-2}$)	0.065 ($P = 0.06$)		0.006 ($P = 0.89$)	0.031 ($P = 0.45$)
LT	−0.062 ($P = 7\ 10^{-2}$)	**−0.096** ($P = 5\ 10^{-3}$)	0.032 ($P = 0.36$)	0.06 ($P = 8\ 10^{-2}$)		−0.00063 ($P = 0.99$)
SER	**−0.154** ($P = 8\ 10^{-6}$)	**−0.594** ($P = 3\ 10^{-16}$)	−0.024 ($P = 0.49$)	**0.095** ($P = 6.3\ 10^{-3}$)	**0.115** ($P = 9.6\ 10^{-4}$)	

Pearson correlations are represented above the diagonal for Vis and below for Korčula. Associated probabilities (two-tailed test) are displayed in parenthesis. Significant ($P < 0.05$) correlations are highlighted in bold.

Effects of Covariates Other Than Sex and Age

In most published studies, age and sex are accounted for in analysis but effects of overall body stature, height, and BMI and YrEd have been less systematically explored.

The effect of each covariate/cofactor on ocular biometrical traits was tested singly in the two Croatian populations and expressed as a percentage of the trait variance explained (Table 3). The single covariate with the strongest effect was similar in both populations: age for refraction (4.3%–11% of the variance), ACD (6.6%–6.5%) and LT (14.9%–14.1%), height for AL (13.2%–7.9%) and CC (11.4%–9.9%), and YrEd for CT (4.4%–1.9%). CT appeared to be the trait the least influenced by any of the covariates tested and BMI the covariate with the least effect (at most explaining 3.6% of the variance in LT in Korčula). Given that the samples studied were adult, sex (rather than sex and age) will be a confounder in the height measures, but the size of the effects were always greater for height than for sex, indicating that height has a specific influence. The effects of multiple explanatory variates were explored further in the best-fitting models.

Heritabilities of Oculometric Traits

Best-fitting sets of explanatory variates and the heritability of traits adjusted for these were estimated by using general linear mixed models (Table 4). In both populations, CT was the ocular trait that was the least affected by any combination of explanatory variates (in the best models: YrEd and BMI explained 2% of CT variance in Korčula; YrEd and age explained 5.6% in Vis) and displayed one of the strongest heritabilities (71.5% ± 12% [SE] in Korčula; 74.8% ± 12% in Vis). Lens thickness was the trait the most affected by the covariates (age, YrEd, and height explaining 17% of its variance in Korčula, age and height 15% in Vis) and displayed moderate heritabilities (31.8% ± 11% in Korčula, 37.5% ± 12% in Vis).

At least 9% of the variance was accounted for by covariates for all other traits, and their heritability, after adjustment for those covariates, varied from high for CC (52.4% ± 12% [SE] in Korčula; 84.1% ± 16% in Vis), AL (73.7% ± 14% in Korčula; 37.9% ± 14% in Vis), and ACD (45% ± 12% in Korčula; 59% ± 15% in Vis) to low for refraction (17.5% ± 9% in Korčula, <1% ± 4% in Vis). Given the standard errors of the estimates, differences in trait heritability in the two cohorts were not statistically significant (two-sided tests on *z*-scores), although they were suggestive for AL ($P = 0.06$) and refractive error ($P = 0.09$).

Quadratic Relationship with Age Fitted Best for ACD and for Refraction in Both Populations

Two of the ocular traits displayed significant or suggestive evidence of heritability differences between sexes in both populations: the most significant, AL (heritability of 84% ± 19% in the women, 9% ± 9% in the men for Korčula; 71.8% ± 23% in the women, 19.5% ± 19% in the men for Vis) and LT (61.9% ± 18% in the women and 11.6% ± 12% in the men in Korčula; 55.9% ± 21% in the women and 9.3% ± 10% in the men in Vis). ACD displayed statistically significant differences in heritability between the sexes in Korčula only (8% ± 8% in the women and 61% ± 25% in the men).

TABLE 3. Single Covariate Effect

Trait	Transformation	Population	Age	Sex	Height	BMI	YrEd
SER	rnk	Vis	**4.3**	0.8	1.8	1.4	2.9
		Korčula	**11**	0.6	2.9	3.1	2.9
AL	rnk	Vis	1.4	7.8	**13.2**	0	2
		Korčula	0.3	6.6	**7.9**	0	1.9
ACD	rnk	Vis	**6.6**	1.6	3.2	0.8	4.1
		Korčula	**6.5**	0.9	2.9	1.6	1.5
CC		Vis	2.8	4.3	**11.4**	0	5.7
		Korčula	1.8	4.7	**9.9**	0	3
CT		Vis	1.2	0.2	0.4	0.3	**4.4**
		Korčula	0.3	0.1	0	0.02	**1.9**
LT		Vis	**14.9**	0.2	0.1	1.8	2.2
		Korčula	**14.1**	0.2	2.7	3.6	0.7

The effect of a single covariate (expressed as % trait variance explained) was estimated in a general linear model, taking family structure into account. SER, AL, and ACD were rank transformed to normality (rnk) before analysis. The strongest contributions per trait are highlighted in bold.

TABLE 4. Covariate Effects and Heritabilities of Ocular Biometric Traits in the Two Croatian Islands

	Best Model			
Trait/Covariates	**Covariate Effect Size**	**Trait Variance Explained by Covariates (%)**	**Heritability h^2 (SE)**	**h^2_{agesex} (SE)**
Vis Island				
SER (rnk)				
Age	0.126	10.9	0.001 (0.001)	0.001 (0.002)
Age^2	−0.001			
Height	−0.011		$P = 0.47$	$P = 0.47$
AL				
Height	0.038	13.2	**0.37 (0.14)**	**0.45 (0.15)**
			$P = 7.6\ 10^{-3}$	$P = 2.2\ 10^{-3}$
ACD (rnk)				
Age	−6.32E-02	9.9	**0.59 (0.15)**	**0.56 (0.15)**
Age^2	0.0004		$P = 8.8\ 10^{-5}$	$P = 1.5\ 10^{-4}$
Sex	0.283			
CC				
Height	0.008	12.8	**0.84 (0.16)**	**0.87 (0.16)**
YrEd	0.011		$P = 1.2\ 10^{-7}$	$P = 3.5\ 10^{-8}$
CT				
Age	−0.269	5.6	**0.75 (0.12)**	**0.76 (0.12)**
YrEd	0.054		$P = 5.6\ 10^{-10}$	$P = 5.3\ 10^{-10}$
LT				
Age	0.013	15.2	**0.37 (0.12)**	**0.36 (0.12)**
Height	0.0033		$P = 1.8\ 10^{-3}$	$P = 2.1\ 10^{-3}$
Korčula Island				
SER (rnk)				
Age	0.095	15.69	0.17 (0.09)	**0.20 (0.09)**
Age^2	−0.0007		$P = 0.07$	$P = 0.025$
Height	−0.0035			
YrEd	0.0226			
AL (rnk)				
Sex	0.2595	9.1	**0.74 (0.14)**	**0.69 (0.13)**
Height	0.0205		$P = 1.56\ 10^{-7}$	$P = 3.03\ 10^{-7}$
YrEd	0.0237			
ACD (rnk)				
Age	−0.062	9.9	**0.45 (0.12)**	**0.41 (0.11)**
Age^2	0.0004		$P = 1.55\ 10^{-4}$	$P = 2\ 10^{-4}$
Sex	0.256			
Height	−0.004			
YrEd	0.0149			
CC				
Age	−0.002	11.42	**0.52 (0.12)**	**0.46 (0.12)**
Height	0.009		$P = 2.1\ 10^{-5}$	$P = 8.4\ 10^{-5}$
YrEd	0.0049			
CT				
BMI	2.39E-01	2.0	**0.71 (0.12)**	**0.72 (0.12)**
YrEd	4.81E-01		$P = 3.6\ 10^{-9}$	$P = 4.5\ 10^{-9}$
LT				
Age	0.014	16.9	**0.32 (0.11)**	**0.34 (0.11)**
Height	0.0003		$P = 4.2\ 10^{-3}$	$P = 1.6\ 10^{-3}$
YrEd	0.005			

Best model represents the best-fitting and most parsimonious model, with significant covariates built after testing the effects of age squared (age^2), sex, BMI, height, and YrEd. SER, AL, and ACD were rank transformed to normality (rnk) before analysis. The heritability of the traits adjusted for age and sex only (h^2_{agesex}) is also reported to allow comparison with published data. Significant ($P < 0.05$) heritabilities are highlighted in bold. Fixed effects are reported as regression coefficients expressed in units of the covariate fitted or men compared to women for sex effect.

Heritability for the traits adjusted for age and sex only are also displayed in Table 4 to allow comparisons with published data. These two variables explained less of the variance of the traits than did the sets from the best models (e.g., explained 7% of CC variance in Korčula and Vis rather than 11–13%).

DISCUSSION

The analysis of ocular biometrical traits in two isolated Croatian insular populations led to consistent results. The measures were in agreement with the ophthalmology literature, with a slightly shorter AL and ACD. Apart from population and sampling differences, the differences could be accounted by the use of different methodologies, an older mean age (56.3 years), or a different ratio of the sexes in our cohorts. In an elderly Norwegian cohort,[30] the reported AL was 23.11 ± 1.23 mm, with female values lower than male values, similar to the values displayed in Table 1. The high positive correlation between AL and CC (0.46–0.44) was similar to the correlation reported in a cross-adult population study in a Sardinian isolate (0.4)[8] and

reflects the fact that in the most common (emmetropic) state, longer eyes tend to have flatter corneas[7] and vice versa, compensating each other for good focus on the retina. The strengths of the correlations between refraction and each simple component were in agreement with refractive development in the myopic range (minus sign SER) being strongly correlated with posterior elongation of the eye: the negative correlation between ACD and SER was lower than that between AL and SER, with relatively little compensatory lens or corneal changes (lower correlations between SER and LT or CT). Pearson correlations between refraction and the biometric components AL, ACD, CC, and lens power, similarly adjusted for age and sex, in a large survey of European 12-year-old children were −0.47, −0.22, 0.09, and 0.08, respectively,[31] and in the same study, AL and CC as well as AL and ACD, showed significant positive correlation, whereas AL and lens power (therefore, LT) showed negative correlation, thus very similar to the Croatian adult measures.

Age and sex influences on these traits are also well documented,[32] and our data are in agreement. When sex and age were fitted as sole effects, the men displayed statistically significant longer eyes, deeper ACD, and flatter corneas, and all traits were influenced by age. In the full models, the men still had significantly longer ACD (in both populations) and AL (in Korčula only) when height was accounted for, although adjustment for height accounted for all sex differences in CC. In full models, age did not influence AL in either of the two Croatian isolates, although age reduced ACD, CT, and CC and increased SER and LT. For refraction and ACD, quadratic, rather than linear, relationships with age were better fits, with a reverse sign of association with age, in agreement with the well-documented opacification of the lens and shift in the hyperopic direction for refraction in older age.

BMI had little influence on the traits analyzed. In contrast, accounting for height and education reduced most trait variances, especially for CC. A recent investigation (the Beaver Dam Eye Study[29]) of the effects of stature and education together with age and sex on three oculometric traits (AL, CC, and ACD) in an adult white population showed similar, although not identical, results, with height and education accounting for all sex differences and attenuating the age effect. Therefore, education and height seem to account, at least partially, for the age and sex effects on ocular traits in an adult population. In an Australian twin study,[20] education attainment explained 4.4% of the variance in refraction, close to the 3% found in our study using another, crude, measure of exposure to near work. It is now clear that many genes with small effects contribute to most sex and age-adjusted height variation,[33] and studies[20,34] have suggested a strong genetic component for education attainment. In future testing of single genetic variant effects on ocular biometric traits, setting models accounting for height and education would increase signals for genes not involved in height or length of education, whereas not fitting them will allow detection of those as well. The fact that sex and height effects can be confounded should also be kept in mind.

Heritabilities (i.e., the proportion of the variance of the covariates_adjusted traits accounted by additive genetic effects) were similar in both Croatian populations. The strongest differences (almost statistically significant) were observed for AL and refraction. This result is in agreement with those traits being the most influenced by environment and the most plastic, and therefore their heritability is the most subject to fluctuation from population to population with possible distinct environmental cues. Substantial to high heritabilities were estimated for all traits but SER, ranging from 32% for LT in Korčula to 84% for CC in Vis, although for SER it was 17% in Korčula and 0.1% in Vis, not significantly different from 0 in either population.

In the published study performed in a Sardinian isolated population,[8] age- and sex-adjusted heritabilities for AL, SER, ACD, and CC were similar to ours, respectively, 45% ± 14% (69% ± 13% Korčula; 44.6% ± 14.6% Vis), 18% ± 16% (20% ± 9.1% Korčula; 0.16% ± 0.2% Vis), 37% ± 17% (41.6% ± 11% Korčula; 56.1% ± 14.8% Vis), and 54% ± 17% (45.6% ± 11.6% Korčula; 87% ± 16% Vis). Heritabilities for all simple oculometric components were, by and large, also comparable to other published datasets across the diverse populations surveyed, with most using a twin design. In contrast, heritability estimates for SER in the Croatian isolates were low, as reported (weak to moderate) for the Sardinian isolate and in family studies when based on parent–offspring correlations[12,35] or families selected on myopic probands,[36] compared with the high heritabilities reported for SER in diverse twin studies.[37] While this paper was under review, the Beaver Dam Eye Study reported a high heritability (0.58 ± 0.13) for SER in its cross-population sample,[38] using all informative relative pairs and a variance component method similar to ours. The wide range of estimates across studies most likely reflects that SER is strongly influenced by the environment, as epidemiologic studies have highlighted.[16,39,40] Parent–offspring or avuncular correlation–based simple estimates assume that the environmental component has not changed within one generation, whereas the estimates based on resemblance between twins assume that the shared environment component is the same for dizygotic and monozygotic twins, and none assume gene–environment interactions. Similar large heritability estimate discrepancies between studies and study designs were noted for IQ, another trait likely to be strongly influenced by environment and gene–environment interactions.

Sex-specific heritabilities were found as reported in the Sardinian isolate study. They were statistically significant for ACD, AL, and LT in Korčula and were suggestive for AL and significant for LT in Vis. However, the heritabilities were in opposite ranks from the ones reported for Sardinia, stronger in the women for AL and stronger in the men for ACD. The sample sizes analyzed in these isolates were similar ($N = 609$ in Sardinia). In sex-separate analyses, the number of same-sex pairs would decrease to low levels where sampling inclusions of discordant pairs, not uncommon for AL, will have a lot of weight. Our divergent results on sex-specific heritabilities thus invite caution regarding the generality of the conclusions reached in these sex-specific analyses and point out that larger cohorts are necessary for clarification.

In conclusion, this study should help establish basic models on which to conduct future QTL mapping studies. It also complements our knowledge on two oculometric traits, CT and LT, that have attracted less attention than AL, which plays a more central role in myopia. The substantial heritabilities of all the simple biometric traits promise good statistical power in future gene-mapping studies and insight into more complex ocular diseases.

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